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ACTH cells of pituitary pars distalis of viscacha (*Lagostomus maximus maximus*): Immunohistochemical study in relation to season, sex, and growth

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Abstract

Corticotrope or ACTH cells were immunohistochemically identified in the pituitary pars distalis (PD) of viscacha by using the polyclonal antiserum against ACTH (1–24). The localization, distribution, shape, percentage immunopositive area, and major cellular and nuclear diameters of these cells were analyzed by image analysis in adult male viscachas captured in their natural habitat during the year and after the chronic administration of melatonin. The same parameters were analyzed in immature male and adult female viscachas. ACTH cells in adult males were mainly localized in the dorsal and cephalic regions of PD. They were isolated, forming small groups and in contact with follicular structures and blood vessels. They were pleomorphic, with some of them being polygonal, oval, round, and others, stellate with cytoplasmic extensions. The percentage immunopositive area and the major cellular diameter showed seasonal variations with lower values during June and July (early winter). A decrease in the percentage immunopositive area was observed after the administration of melatonin in adult male animals. ACTH cells of immature animals differed from the adults' cells in their distribution, shape, pattern of immunolabeling, and percentage immunopositive area. These parameters in adult females did not vary in relation to adult males at the same time of the year although. However, the cells in females were smaller in size during April–May. In pregnant viscachas (June–July), these parameters did not show significant differences with the results of non-pregnant females (April–May). This suggests that the environmental stressors do not exert the same influence on the hypothalamo–pituitary–adrenal axis in adult male and female viscachas, probably due to the physiological changes caused by pregnancy. Our results in adult male viscacha demonstrate that the morphology of the ACTH cells varies according to the different seasonal conditions, thus participating in the adaptation process of this rodent to the environment. The elevated levels of melatonin during winter months might inhibit the synthesis of ACTH, probably when affecting some secretagogue of this pituitary hormone. Moreover, the morphological variations observed between adult and immature male viscachas and between both sexes suggest that the steroid gonadal hormones might act on the development and activity of ACTH cells.

Keywords: *Lagostomus maximus maximus*; Pituitary gland; ACTH cells; Immunohistochemistry; Season; Sex; Growth

1. Introduction

Several species maintain a dynamic equilibrium with the environmental signs of their natural habitat to achieve survival. Stress threatens this equilibrium and the adaptation to it gives a survival advantage. The stressors activate the hypothalamo–pituitary–adrenal axis (HPA) and the sympathoadrenal system. The HPA axis stimulation is characterized by the activation of corticotropin-releasing factor (CRF) and arginine-vasopressin (AVP) neurons, which in turn, stimulate the biosynthesis of pro-opiomelanocortin (POMC) and release of ACTH from corticotropes of the pituitary pars distalis (PD) (Engler et al., 1989; Romero and Sapolsky, 1996).

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The ACTH released into the peripheral circulation in response to stress stimulates the synthesis and secretion of adrenal glucocorticoids (Aguilera, 1994; Trainer et al., 1995). Studies carried out with rats demonstrated that the feedback effects between some hypothalamic peptides (CRF, AVP) and adrenocortical steroids influence the morphology, size, and number of pituitary ACTH cells (Childs, 1992; Westlund et al., 1985). Besides, the gonadal steroids may also affect these cells (Melendowicz et al., 1987).

Tanaka and Kurosumi (1986) classified the corticotropes or ACTH cells in rats into three types according to their morphology, distribution, and content of the cytoplasmic granules: (1) typical corticotropes, stellate in form with secretory granules aligned in the cellular periphery; (2) atypical corticotropes, polygonal in shape and with a great quantity of secretory granules; and (3) intermediate corticotropes, with a small number of secretory granules, most of them containing α-MSH.

It has been reported that the corticotropes show variations during different stages of the reproductive cycle in bat (Singh and Krishna, 1997) and in ewe (Hifny et al., 1982). Vidal et al. (1995) demonstrated that in mink (*Mustela vison*) there are differences in the average area occupied by ACTH cells between male and female animals. Morphological and functional changes were observed during the maturation of the HPA axis. In studies carried out with the sheep pituitary, at least two types of cells, named fetal and adult, were described. Moreover, the number of corticotropes decreases from gestation to adulthood in the anterior pituitary (Mulvogue et al., 1986; Perry et al., 1985). However, a continuous increase of the area occupied by the ACTH cells from birth to adulthood was reported in mink (Vidal et al., 1995).

There is evidence indicating that the pineal gland exerts the photoperiodic regulation of some pituitary functions through melatonin. Seasonal variations in the cytomorphology, intensity of immunolabeling, and cytoplasmic granulations of the corticotropes throughout the year were described in frogs, which probably influence vitellogenesis and reproduction processes (Pramoda and Saidapur, 1991). It has also been shown that the postnatal increase in the area of ACTH cells in Djungarian hamster under long days was inhibited in short-days-exposed or melatonin-treated animals (Hira et al., 2001).

Some investigations have shown that the neuroendocrine response to various stressors is attenuated during gestation in rat (Neumann et al., 1998), sheep (Keller-Wood, 1994), and human (Schulte et al., 1990) and also during lactation in rat (da Costa et al., 1996). Other authors have demonstrated that the hyporesponsiveness to stress during pregnancy is postulated to be a protection mechanism for the fetus, because the increased maternal HPA axis activity has been related with behavioral (Weinstock, 1997) and endocrine (Fameli et al., 1994) problems in the offspring.

The *Lagostomus maximus maximus* (viscacha) is a rodent mammal with nocturnal habits that live in underground caves in semiarid zones in the center of Argentina. The male viscacha exhibits seasonal variations in its annual reproductive cycle and in other metabolic functions that are under the control of day length. Its reproductive activity occurs during the long days of summer and early autumn whereas during the short winter days, these animals experience an important testicular regression with the subsequent ceasing of the reproductive activity (Aguilera-Merlo et al., 2005; Filippa et al., 2005; Fuentes et al., 1991, 1993; Muñoz et al., 1997, 2001). Besides, during winter the secretory ability of the pineal gland is increased (Dominguez et al., 1987) and maximum levels of melatonin in blood were determined (Fuentes et al., 2003).

The environmental signs determine the beginning or end of the specific seasonal adaptations to maintain a positive energetic balance. In this way, the viscacha can adapt physiologically to the climatic seasons, performing the necessary endocrine adjustments to achieve survival and to increase the reproductive success. Environmental winter conditions such as short photoperiod, low temperature, reduced hydric availability, and variations in the composition of food are the stressors that provoke the activation of the HPA axis. The adrenal gland is an organ important for the seasonal adaptations of this photoperiodic wild life rodent. Previous investigations in adult male viscachas have shown morphological and biochemical seasonal changes in the adrenal cortex (Ribes et al., 1999). Some characteristics of reproductive organs (Weir, 1971), pregnancy, and lactation have been studied in the female viscacha (Weir, 1974; Weir and Rowlands, 1974). The most critical stage of the reproductive physiology is the period from the end of pregnancy to the end of lactation during which mother and offspring are more vulnerable to the adverse effects of the environment. The viscacha has adapted to the seasonal climatic variations developing a long pregnancy during winter (145–166 days; Mossman and Duke, 1973) and giving birth at times with optimal temperature conditions and food and hydric availability (spring-summer).

On the basis of the results described for the different species and of our previous investigations, the purpose of this work was to immunohistochemically identify ACTH cells of PD in adult male viscachas throughout the year and after melatonin administration. Moreover, ACTH cells were studied in relation to the animal's sex and age. The morphometric parameters analyzed in this study, percentage immunopositive area, and major cellular and nuclear diameters have been considered as a measure of the cellular activity (Vidal et al., 1995).

2. Materials and methods

Adult viscachas of both sexes, immature and prepubertal males were captured in their natural habitat near San Luis, Argentina (33° 20′ south latitude, 760 m altitude) during 2004. The capture was realized using traps placed in their burrows. The reproductive condition (immature, prepubertal, and adult) of viscachas was carefully assessed on the basis of observations by light microscopy of testes and ovaries, and additionally, on the basis of body weight (Branch et al., 1993; Llanos and Crespo, 1954; Mohamed et al., 2000). Values of solar irradiation expressed as heliophany

and monthly mean values of precipitations and temperature were provided by the Servicio Meteorológico Nacional San Luis. In June (early winter), the lowest values of heliophany (H), precipitation (P), and temperature (T) were observed.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
H	11.3	10.56	8.20	7.78	6.40	5.63	6.60	7.04	7.39	7.98	10.20	11.07
P (mm)	112	88	92	42	12	10	12	10	20	38	79	100
T (°C)	24	21	18	15	11	9.50	11	13	16	20	23	25

After captured, animals were immediately taken to the laboratory, anesthetized with Nembutal (pentobarbital), and killed by decapitation. The brain was rapidly exposed and the pituitary gland was excised, sagittally sectioned and processed for light microscopy, fixed in Bouin's fluid, embedded in paraffin, and serially sectioned in the horizontal plane.

Administration of melatonin: eight adult male viscachas captured during the month of February (summer) were used. The rodents were kept in isolated boxes with free access to water and food at 20 ± 2 °C. They were maintained under a 14L:10D photoperiod. The experimental group received two daily subcutaneous injections of melatonin (Sigma, 100 μg/kg body weight in oil solution) at 09:00 and 17:00 h, during 9 weeks. The control group received only the diluent.

2.1. Immunohistochemical techniques

Serial sagittal pituitary sections (5 μm thick) were cut and carried through xylene and graded alcohols to water. Slides were incubated for 20 min in a solution of 3% H_2O_2 in water to inhibit endogenous peroxidase activity. Then, they were rinsed with distilled water and phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Non-specific binding sites for immunoglobulins were blocked by incubation for 15 min with 0.25% casein in PBS and rinsed with distilled water and PBS. Sections were then incubated for 60 min in a moist chamber at 4 °C with the primary antiserum raised in rabbits against ACTH (1–24) (polyclonal; BioGenex, San Ramon, USA). After they were rinsed with PBS for 10 min, the immunohistochemical visualization was carried out using the Super Sensitive Ready-to-Use Immunostaining Kit (BioGenex, San Ramon, USA) at 20 °C. The biotin–streptavidin amplified (B-SA) system was used as follows. Sections were incubated for 30 min with diluted biotinylated anti-rabbit IgG, and after being washed in PBS, they were incubated for 30 min with horseradish peroxidase-conjugated streptavidin, and finally washed in PBS. The reaction site was revealed by 100 μl of 3,3′-diaminobenzidine-tetrahydrochloride (DAB) chromogen solution in 2.5 ml PBS and 50 μl H_2O_2 substrate solution. The slides were counterstained with hematoxylin for 1 min, dehydrated, and mounted. In all cases, two controls for specificity of the primary antibody were made: (1) omission of primary antibody, and (2) absorption of primary antibody with homologous antigen.

2.2. Morphometric study

Computer-assisted image analysis system was used to measure the immunopositive area of ACTH cells from pituitaries at different levels. The system consisted of an Olympus BX-40 binocular microscope (magnification, 400×), interfaced with a host computer, image processing and recording system. The images were captured by a Sony SSC-DC5OA camera and processed with Image Pro Plus 5.0 software under control of a Pentium IV computer. The software allowed the following processes: image acquisition, automatic analogous adjust, thresholding, background subtraction, distance calibration, area and diameter measuring, and diskette data logging. The image was displayed on a color monitor, and the immunopositive areas were measured with the image analysis system. Before counting, a standard area of 18141.82 μm^2 was defined on the monitor, and distance calibration was performed using a slide with a micrometric scale for microscopy (Rechert, Austria). Microscopic fields not entirely occupied by the tissue were not analyzed. The percentage immunopositive area (% IA) of ACTH cells was calculated using the formula % IA = Ac/At × 100, in which Ac was the area of immunolabeled cells and At was the pars distalis area per microscopic field. The light microscope images of 80 microscopic fields per section (four serial sections per pituitary gland) were evaluated. Four pituitary glands were used per group in the study in relation to season, sex and growth. Finally, 1280 measurements were performed per group. Four pituitary glands for both groups of the administration of melatonin (experimental and control) were used, and 1280 measurements per group were performed. The major cellular and nuclear diameters were measured using the length tool of the Image Pro Plus software on each ACTH cells with a visible nucleus. These diameters were measured for 50 immunoreactive ACTH cells per group. The results were expressed as means ± standard error of the mean (SEM) for all data sets.

All data of the different groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test. Differences between experimental and control groups were evaluated using Student's t test. A probability of less than 0.05 was assumed to be significant.

3. Results

The *Lagostomus maximus maximus* pituitary PD exhibited different regions or zones called ventral (anterior) and dorsal (posterior, close to Rathke's pouch). Besides, two extremes were distinguished: a cephalic one (superior, connected with PT) and a caudal one (inferior) (Fig. 1A).

In adult male viscachas captured in their habitat, the ACTH cells were distributed throughout the parenchyma of PD, though they were mainly concentrated in the cephalic extreme and dorsal region (Fig. 1A). These cells were generally isolated or forming small groups of three and five cells. ACTH cells were found either in contact with the colloidal lumen or as part of the follicle in a basal position, without contacting the lumen. They presented a marked pleomorphism, being some of them polygonal, round or oval with a voluminous cytoplasm, and others, stellate with long cytoplasmic processes, generally extending towards blood vessels or surrounding non-immunolabeled cells (Figs. 1B and C). The nucleus was round or oval, centrally placed. The localization of the immunolabeling in the cytoplasm changed significantly. In cells localized in the cephalic and dorsal regions, secretory granules were observed in the perinuclear region and in the periphery of the cellular cytoplasm. On the contrary, cells localized in the ventro-medial and caudal zones exhibited immunolabeling throughout the cytoplasm. The percentage immunopositive area and the major cellular diameter changed throughout the year. These parameters decreased significantly ($P < 0.001$) during June–July (early winter, Figs. 1D and E) in relation to the previous bimester. However, no variations were observed in the nuclear diameter. The percentage area increased significantly ($P < 0.001$) during the following 2 months (August–September) whereas the increase of the major cellular diameter was slower. During October–November, a significant increase ($P < 0.001$) was observed in this parameter (Table 1, Figs. 1F–H).

The values of the parameters studied in viscachas administered with melatonin were similar to those found during June–July (short photoperiod, early winter). However, a decrease in the percentage area occupied by ACTH cells was observed in the experimental group in relation to the control group ($P < 0.05$). No variations were observed in the nuclear and cellular diameters (Table 2).

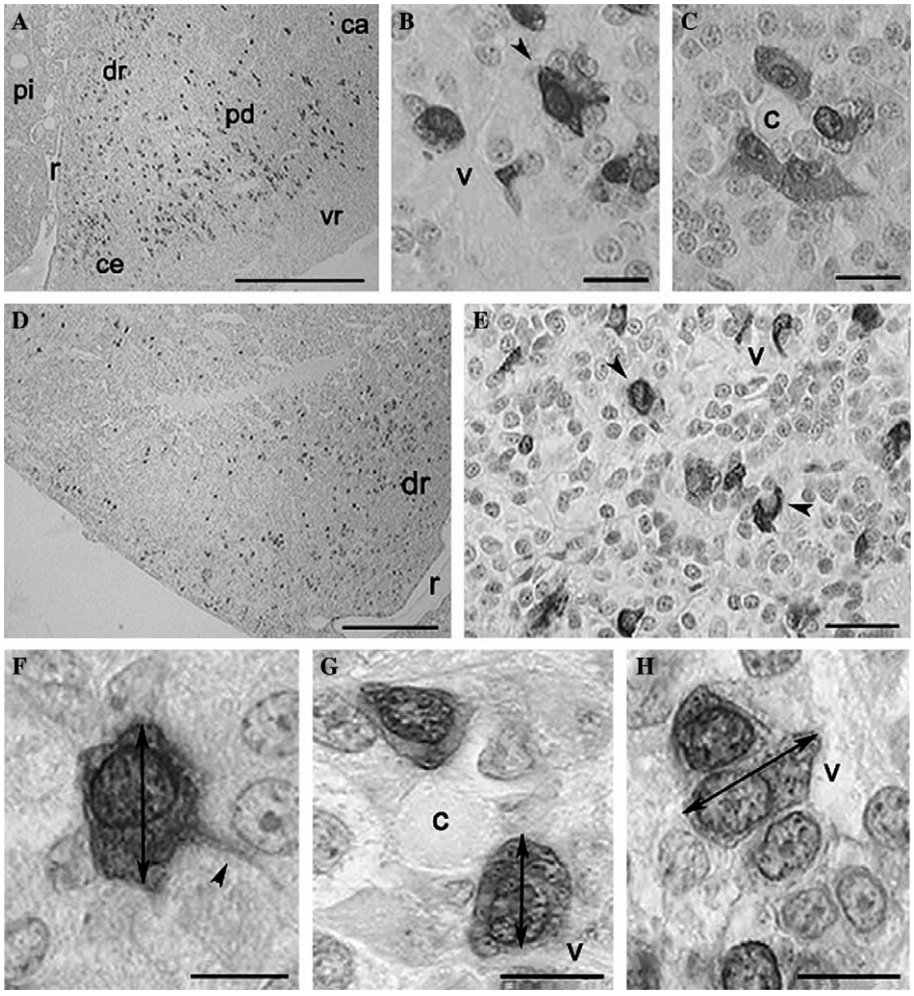

Fig. 1. (A–H) Pituitary glands of adult male viscachas captured during April (A, B, C, and F), July (D, E, and G), and November (H). (A) ACTH cells are concentrated in the cephalic extreme and dorsal region. pd, pars distalis; pi, pars intermedia; r, Rathke's pouch; ce, cephalic extreme; ca, caudal extreme; dr, dorsal region; vr, ventral region. Scale bar, 500 μm. (B) ACTH cell oval in shape in contact with a blood vessel (v) and ACTH cell with long cytoplasmic processes extending towards a blood vessel are apparent (arrowhead). Scale bar, 12.5 μm. (C) ACTH cells either appeared in contact with the colloidal lumen (c) or are part of the follicle in a basal position, without contact with the lumen. Scale bar, 12.5 μm. (D) A small number of ACTH cells is present in the dorsal region (dr) of pituitary PD; r, Rathke's pouch. Scale bar, 500 μm. (E) Pleomorphism of ACTH cells, being some of them polygonal or oval, and others, stellate with long cytoplasmic processes, extending towards blood vessels (v) or surrounding non-immunolabeled cells (arrowheads). Scale bar, 25 μm. (F) ACTH cell with irregular morphology extended a thin cytoplasmic process between other adjacent cells (arrowhead). (↔) Major cellular diameter. Scale bar, 10 μm. (G) ACTH cells in contact with the colloidal lumen (c) and a blood vessel (v). (↔): major cellular diameter. Scale bar, 10 μm. (H) ACTH cells forming a small group along the surface of blood vessel (v). (↔) Major cellular diameter. Scale bar, 10 μm.

Table 1
Percentage immunopositive area, major cellular and nuclear diameters of ACTH cells of adult male viscachas: seasonal study

	Feb–Mar ($n=4$)	Apr–May ($n=4$)	Jun–Jul ($n=4$)	Aug–Sep ($n=4$)	Oct–Nov ($n=4$)
Immunopositive area	3.23 ± 0.25	3.19 ± 0.09	$1.86 \pm 0.16^{***}$	$3.41 \pm 0.17^{***}$	3.21 ± 0.10
Major cellular diameter	16.09 ± 0.33	15.64 ± 0.10	$12.92 \pm 0.15^{***}$	13.88 ± 0.18	$15.28 \pm 0.04^{***}$
Nuclear diameter	6.52 ± 0.07	6.24 ± 0.14	6.17 ± 0.25	6.10 ± 0.10	6.27 ± 0.15

Note. The immunopositive area is expressed as mean ± SEM (%). The major cellular and nuclear diameters are expressed as means ± SEM (μm). The significant differences were determined by analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test.
*** $P < 0.001$ compared with the value of the previous period.

The localization, distribution, morphological characteristics, immunolabeling pattern, and percentage immunopositive area of ACTH cells in immature male viscachas (1.0–2.0 kg body weight) showed the following differences in relation to what was observed in adult males: (1) they were widely distributed throughout the PD parenchyma (Fig. 2A); (2) they were mainly oval, pyramidal or round with a central nucleus (Fig. 2B); (3) immunolabeling was generally homogeneous throughout the cytoplasm; (4) the percentage immunopositive area was significantly higher

Table 2
Percentage immunopositive area, major cellular and nuclear diameters of the ACTH cells of melatonin-administered adult male viscachas

	Control group ($n=4$)	Experimental group ($n=4$)
Immunopositive area	1.94 ± 0.06	1.42 ± 0.02*
Major cellular diameter	14.86 ± 0.27	14.38 ± 0.28
Nuclear diameter	6.11 ± 0.07	6.14 ± 0.07

Note. The immunopositive area is expressed as mean ± SEM (%). The major cellular and nuclear diameters are expressed as means ± SEM (μm). The significant differences were determined by Student's *t* test.
* $P < 0.05$ compared with the value of the control group.

($P < 0.05$). The values of major cellular and nuclear diameters did not show significant differences when comparing the immature male with adult male viscachas of the same time of the year. The male viscachas weighing between 3.0 and 4.0 kg (prepubertal) exhibited a more varied cellular pleomorphism, differently from the previous group. Oval, pyramidal, and round cells and stellate cells with evident cytoplasmic processes were distinguished. The cytoplasmic immunolabeling exhibited similar characteristics to the pattern observed in adults (Table 3).

ACTH cells of adult female viscachas were found in all the PD, mainly concentrated in the dorsal region of the parenchyma (Fig. 3A). A great quantity of these cells was close to follicular structures in contact with lumen or as being part of the follicular structure. ACTH cells were polygonal, oval or round in shape. Very few cells exhibited cytoplasmic prolongations. The cytoplasmic immunolabeling was homogeneous; however, some cells localized in the ventral region exhibited immunolabeling in the periphery of the cellular cytoplasm (Fig. 3B). The distribution, shape, pattern of immunolabeling, and percentage immunopositive area of ACTH cells in females did not vary in relation to adult males at the same time of the year although. However, it was observed that ACTH cells of non-pregnant females were smaller in relation to adult males ($P < 0.05$) during April–May. The percentage immunopositive area and the major cellular and nuclear diameters did not show significant variations between non-pregnant (April–May) and pregnant females (June–July; Table 4).

4. Discussion

The *Lagostomus maximus maximus* is a rodent with nocturnal habits and seasonal reproduction. Its reproductive activity occurs during the long days of summer and early autumn whereas during the short winter days, these animals experience an important testicular regression with the subsequent ceasing of the reproductive activity (Aguilera-Merlo et al., 2005; Filippa et al., 2005; Fuentes et al., 1991, 1993; Muñoz et al., 1997, 2001). The sunlight is the most predictable signal that synchronizes this and other physiological processes. The pineal gland through melatonin provokes the behavioral response according to the variations in day length. The cellular activity of pinealocytes and the values of serum melatonin are at the maximum during July and August (Dominguez et al., 1987; Fuentes et al., 2003).

The pituitary–adrenal cortex system is part of a general adaptation mechanism of several animals under extreme

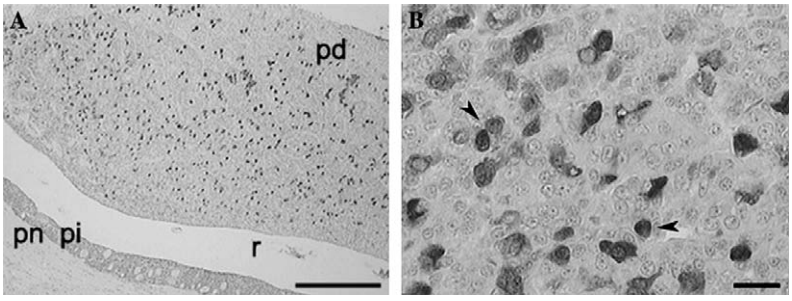

Fig. 2. (A and B) Pituitary gland of immature male viscacha. (A) ACTH cells are widely distributed throughout the PD parenchyma. pd, pars distalis; pi, pars intermedia; pn, pars nervosa; r, Rathke's pouch. Scale bar, 500 μm. (B) ACTH cells are mainly oval, pyramidal or round and its immunolabeling is homogeneous in all the cytoplasm (arrowheads). Scale bar, 25 μm.

Table 3
Percentage immunopositive area, major cellular and nuclear diameters of the ACTH cells of male viscachas of different ages according to their body weight

Body weight (kg)	1.0–2.0 ($n=4$, I)	3.0–4.0 ($n=4$, P)	5.0–7.0 ($n=4$, A)
Immunopositive area	4.97 ± 0.25	4.86 ± 0.54	3.41 ± 0.17*
Major cellular diameter	13.05 ± 0.44	13.92 ± 0.25	13.88 ± 0.16
Nuclear diameter	6.13 ± 0.23	6.13 ± 0.15	6.10 ± 0.10

Note. The immunopositive area is expressed as mean ± SEM (%). The major cellular and nuclear diameters are expressed as mean ± SEM (μm). I, immature animals; P, prepubertal animals; A, adult animals.
The significant differences were determined by analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test.
* $P < 0.05$ compared with the value of the previous groups.

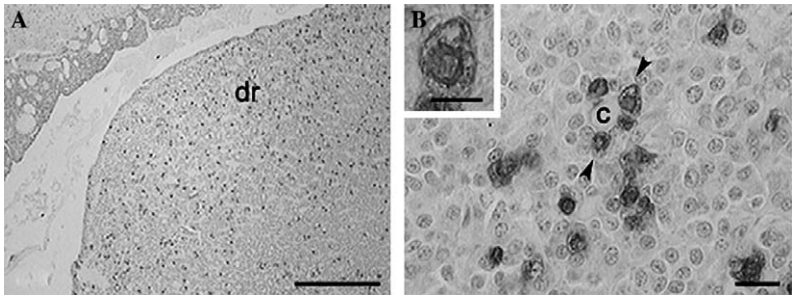

Fig. 3. (A and B) Pituitary gland of adult female viscachas. (A) ACTH cells are concentrated in the dorsal region (dr) of the PD parenchyma. Scale bar, 500 μm. (B) Numerous ACTH cells (arrowheads) are in contact with the colloidal lumen (c). Scale bar, 25 μm. Left inset: higher magnification of an ACTH cell with immunolabeling in the cellular periphery. Scale bar, 10 μm.

Table 4
Percentage immunopositive area, major cellular and nuclear diameters of the ACTH cells of adult male and female viscachas

	April–May		June–July	
	Male ($n = 4$)	Female ($n = 4$, NP)	Male ($n = 4$)	Female ($n = 4$, P)
Immunopositive area	3.193 ± 0.09	2.745 ± 0.17	1.86 ± 0.16	2.32 ± 0.11
Major cellular diameter	15.64 ± 0.10^{a}	14.16 ± 0.30^{b}	12.92 ± 0.15	13.52 ± 0.18
Nuclear diameter	6.24 ± 0.14	5.9 ± 0.13	6.17 ± 0.25	6.26 ± 0.19

Note. The immunopositive area is expressed as mean ± SEM (%). The major cellular and nuclear diameters are expressed as mean ± SEM (μm). NP, non-pregnant; P, pregnant. The significant differences were determined by analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test.
a vs. b, $P < 0.05$.

survival conditions. During winter, viscachas live under certain environmental conditions such as short photoperiod, low temperature, hydric restriction, and food variations (Bontti et al., 1999; Branch et al., 1994). These stressors are responsible for the increase in the glucocorticoids synthesis represented by the high serum levels of corticosterone found in winter in adult male viscachas (Ribes, 2000). Besides, studies of the fasciculata zone of the adrenal cortex demonstrated that the organelles involved in the synthesis of the steroid hormones were well developed during February–March (summer). On the contrary, a cellular exhaustion was observed during August after the synthesis and release of glucocorticoids (Ribes, 2000; Ribes et al., 1999). In rat, it was observed that a continuous stress provoked by water deprivation caused an increase in the corticoid levels which reached the maximum just before the stress ended (Johnson and Levine, 1973). In sand rat, it was reported that during winter the adrenocortical sensitivity to ACTH was incremented, which permitted a higher production of cortisol, and the serum levels elevated until the beginning of spring (Amirat and Brudieux, 1993).

In most of the species, the corticotropes were very heterogeneous as regards parameters of size, shape, secretion, and hormonal storage. In sciaenid teleosts (Yan and Thomas, 1991), hermaphroditic teleost (Ferrandino et al., 2000), white seabrean (Segura-Noguera et al., 2000), *Bufo arenarum* (Miranda et al., 1996), *Chalcides chalcides* (Ferrandino et al., 2001), and in mammals such as rat (Nakane, 1970) and mink (Vidal et al., 1995), ACTH cells showed a precise localization in the parenchyma of PD. In fishes, ACTH cells were described as oval and round, with sizes inferior to 10 μm (Ferrandino et al., 2000; Yan and Thomas, 1991). In reptiles, those cells appeared in groups, isolated, oval and with marked cytoplasmic density (Ferrandino et al., 2001). On the contrary, in rats (Childs et al., 1989; Inoue and Hagino, 1984; Yoshimura and Nogami, 1981), in mink (Vidal et al., 1995), and in our present work with viscacha, the population of ACTH cells was pleomorphic since polygonal, oval, and round cells and stellate cells with evident cytoplasmic prolongations can be observed. Besides, in viscacha, the cells of the cephalic and dorsal regions exhibited a different pattern of cytoplasmic immunolabeling in relation to the cells of the ventral region. These results suggest that there might exist subpopulations of corticotropes that respond to different stimuli. In rat, a decrease in the quantity of secretory granules of the ACTH cells was observed after the animals were subjected to an ether stress. However, these cells kept well granulated after neurogenic stress (Moriarty and Moriarty, 1973). Other investigations have proposed the existence of a population of ACTH cells that in particular circumstances might contain insufficient quantities of hormones for their detection. This suggested a subdivision of the corticotropes in secreting cells and in other cells that are in a stand-by situation, both in a different state of reactivity or in a different stage of the secretory cycle (Childs, 1991, 1992).

In the PD of viscacha, it was frequently observed that the cytoplasmic prolongations were in contact with blood vessels. Besides, two types of colloidal accumulations were described: those corresponding to the typical follicles and those corresponding to pseudofollicles (Mohamed et al., 2000). Some corticotropes or cytoplasmic prolongations

were observed close to the colloidal lumen. These results suggest that the colloid of these structures may have a storage function for the polypeptidic hormones or may serve as a mechanism of protein transport. In rat, Itoh et al. (2000) observed ACTH cells and the network of microvessels, clearly describing that these cells were polygonal in shape and had long cytoplasmic processes ending on the capillaries.

Different studies have established a correlation between the morphological changes of the ACTH cells and their functional state. Investigations carried out with rats demonstrated that after the infusions of CRF, there was an increase of the concentration of serum ACTH and of the cellular area of the pituitary corticotropes. However, Westlund et al. (1985) found an apparent decrease in the percentage of labeled cells, probably due to degranulation. Other investigators observed an increase in the area of the ACTH cells in rats subjected to cold stress (Sasaki et al., 1990). Some authors reported seasonal changes of the secretory activity of ACTH cells in frogs, which have a possible influence on the vitellogenesis and the reproductive activity. Moreover, it was demonstrated that the corticotropes play an important role in the acclimatization processes to new environmental conditions in this species (Gracia-Navarro et al., 1986; Pramoda and Saidapur, 1991).

The results observed in adult male viscachas show seasonal changes in the percentage immunopositive area and cellular diameter of the corticotropes of PD. These parameters are at the maximum during summer, they start to decrease from April–May, and they are at the minimum during June–July (early winter). This suggests that many ACTH cells remained totally or partially degranulated after secreting ACTH, preparing the organism for the different environmental winter conditions. This pituitary hormone stimulates the synthesis and release of adrenal glucocorticoids that perform the metabolic adjustments adequate for survival. The values of the parameters studied were recovered during August–September suggesting that the degranulated cells start to store secretory granules with ACTH again and slowly increase their size. During October–November there are no variations in the percentage immunopositive area in relation to the previous bimester. However, a significant increase of the cellular size is observed, suggesting that the cells synthesize and store great quantities of ACTH hormone.

In melatonin-administered rats, a significant attenuation of the adrenocortical secretory response to acute and chronic stress was observed, with significantly lower hypothalamic CRF levels. These results demonstrated that melatonin attenuates the adrenocortical response to stress influencing the biosynthesis and the secretion of glucocorticoids by affecting the hypothalamic secretagogues of ACTH (Konakchieva et al., 1997). It was reported that the increase in the area of ACTH cells in Djungarian hamster during long days was inhibited during exposition to short days and because of the chronic treatment with melatonin. Therefore, the short photoperiod can suppress, via melatonin, the development of ACTH cells (Hira et al., 2001). In the adult male viscacha, the chronic administration of melatonin provokes a significant decrease of the percentage immunopositive area in the experimental group in relation to the control. The values of all the parameters in melatonin-administered viscachas are similar to those obtained during June–July. This suggests a direct or indirect melatonin action on the activity of the ACTH cells. In these rodents of wild habits, captivity probably provokes a stress that affected the experimental group as well as the control group.

The study carried out in relation to age revealed that there are differences between the immature and adult male viscachas in the distribution, morphology, pattern of immunolabeling, and percentage immunopositive area of the ACTH cells. The immature and prepubertal male viscachas exhibit higher immunopositive area than the adults of the same time of the year whereas their cells are similar in size. This suggests that the quantity of ACTH cells is higher in these animals and decreases towards adult and sexually matured animals. Besides, it has been observed that in adult viscachas there are stellate cells with cytoplasmic prolongations and cells with secretory granules peripherally distributed. In contrast with our results, Vidal et al. (1995) have reported that the size and volume density of the ACTH cells in mink increased from birth to adulthood as a consequence of the stimulatory effect of the gonadal steroids. Various results about the influence of the gonadal hormones on the activity of the ACTH cells have been reported. In rat, it has been observed that the corticotropes decreased after the gonadectomy, but they were restored after the administration of testosterone and estradiol (Melendowicz et al., 1987). However, another study carried out in gonadectomized adult rat demonstrated the inhibitory effect of testosterone on the corticotropes activity. This gonadal hormone might increase the feedback action of the glucocorticoids affecting the secretion of ACTH and/or the processing of POMC (Viau and Meaney, 2001).

Dada et al. (1984) have reported that in adult male and female rats the quantity and volume density of ACTH cells were similar in both sexes. Other researchers have proved that the gonadal steroids act regulating the synthesis and secretion of secretagogues that are responsible for the control of the ACTH cells function (Viau and Meaney, 1991). Studies carried out in male and female rats demonstrated that the response to ether stress was higher in the females due to the stimulatory effect of estradiol. Besides, it was observed that testosterone as well as estradiol provoked the restoration of the ACTH cells activity (Lesniewska et al., 1990). It was also demonstrated that the altered HPA axis response to a stressor stimulus in rat was partially due to changes at the level of the pituitary corticotropes. These cells secreted less quantity of ACTH in response to CRF in vivo as pregnancy advanced and during lactation, thus demonstrating that the corticotropes response to CRF during pregnancy was reduced, probably as a consequence of the decrease of the CRF receptor binding (Neumann et al., 1998). Ma et al. (2005) reported that the content of CRF in

the median eminence was reduced during pregnancy. Therefore, the decreased secretion of this secretagogue accounts for the attenuation of the response to stress in pregnant rat.

In viscacha, ACTH cells of females were significantly smaller in relation to adult males during April–May. However, they exhibited similar percentage immunopositive areas. This suggests that ACTH cells are more numerous in females than in males, probably due to the influence of the gonadal steroidal hormones. The studied parameters do not show any significant differences between non-pregnant and pregnant females (April–May and June–July, respectively). This demonstrates that the environmental stressors do not exert the same influence on the HPA axis in adult male and female animals, probably due to the physiological changes caused during pregnancy.

In conclusion, ACTH cells of viscacha PD change morphologically in relation to the season in response to the different environmental conditions, thus participating in the adaptation process of this rodent. These cells remained totally or partially degranulated after secreting ACTH, preparing the organism for the different environmental winter conditions. This pituitary hormone stimulates the synthesis and release of adrenal glucocorticoids that perform the metabolic adjustments adequate for survival. The elevated levels of melatonin determined during the winter months might inhibit the synthesis of ACTH, probably by affecting some secretagogue of this hypophysial hormone. The variations observed in relation to sex and growth suggest that the steroidal gonadal hormones might act on the development and activity of the ACTH cells.

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